
<< Reading: NASA.TXT;7 8939[7]
4,30-JUL-77 00:36:36,31-JUL-77 15:30:30,31-JUL-77 15:34:39

. . . .

Research plans 1977-78.

A. Microbiological and genetic studies

For several years, the main focus of microbiological research in this laboratory has been on the construction of simple model systems for the genetic diversification of simple genetic codes. This work entailed the use of 'recombinant DNA' technology to construct bacteria that had simple monotonous synthetic sequences, whose further evolution could then be studied in detail. The technical task has proven to be far more difficult than was originally envisaged; but on the way we have made a number of important advances in a) methods of DNA segmentation and implantation, b) understanding of the conditions for expression of genes exchanged among widely diverse bacterial species, and c) the prevalence of mechanisms for promiscuous mixing of genes among bacteria believed to be quite separate in an evolutionary sense -- e.g. Bacillus and Staphyloccus. In a sense this last finding may unexpectedly prove to be the most important of our contributions to the theme of the origin and evolution of life.

In the light of the unexpected ferocity and hostility of contention about the public health hazards of work with recombinant DNA,
-- although we have endeavored to stay as far as possible from situations with credible pathogenic potential -- we have decided to phase out our work under NASA support that directly involves recombinant DNA; and this will no longer be part of our continuation proposal. However, we will comtinue efforts along the lines of c) above, namely to seek still broader understanding of the extent to which genetic information is shared with a common gene pool among microbes. Julian Davies, for example, has long speculated that antibiotic-producing streptomycetes are the primary reservoir of plasmids that recur in a wide range of pathogenic bacteria, and by conferring resistance to the antibiotics result in serious clinical problems. However, direct support for this degree of panmixis has yet to be achieved experimentally. In the course of our observations of the Staphylococcus x Bacillus exchanges mentioned above, we believe we may have some hints how to overcome these experimental barriers, and will apply these ideas to building general "plasmid-traps" to capture the movement of plasmids among a variety of species in their natural soil habitats. The insight sought here would of course be of the greatest importance for a) the theory of evolution of microbes (if not eukaryotes as well); b) practical problems of the sources of antibiotic-resistance; and c)

common-sense tempering of regulatory controls that have become a serious hindrance to research in this field.

This shift of emphasis to natural habitats is coupled with an effort to get a better understanding of the role of solid surfaces in microbial physiology, ecology and genetics. Some years ago, in connection with studies that helped to prove that penicillin acted as an inhibitor of cell-wall synthesis, I was impressed by the role that solid-agar had in controlling the morphogenesis of spheroplasts or L-forms in E. coli. Knowing that many, perhaps most, of the microbes resident in soils have never been isolated in pure culture, I wondered whether much of laboratory microbiology -- using homogeneous liquid media -- is not an artefact, far removed from conditions of the natural habitat. In the interval, many soil microbiologists have made similar observations (summarized in Marshall's book), at least of the influence of interfaces on the growth and physiology of particular species. The most extreme speculation that I propose to investigate is whether there are not many species that have an absolute requirement for a solid substrate -- from which it would follow that conventional methods have been actively selecting against the recognition of an important part of our biosphere. An even more interesting proposition is that these organisms may also include representatives of the simplest, most primitive categories that could give further clues about the baffling missing links in the 'Scala Naturae'. Some (feeble) support for such a speculation can be gathered from the fact that Mycoplasmas, which have the smallest genome-sizes of any presently known freeliving organisms, lack a rigid cell wall. Some of these have, to be sure, been cultivated in suspension, but often only with difficulty; and analogous L-phases of larger bacteria generally require an agar substrate. The implication is that a rigid wall, and the ability to proliferate readily in suspension, are later evolved attributes of the 'typical' bacteria compared to more primitive forms. So far, the most primitive mycoplasmas still have the complete repertoire of the genetic code, and therefore can give us little insight into its primary evolution (unless one takes this as evidence of special creation or of panspermia!).

We must concede that we have made little progress since Oparin in bridging the hiatus between the chemosynthesis of nucleotides and their assembly into meaningful, autocatalytic sequences -- in part for lack of observable representatives of the intermediate stages.

While this would be the most rewarding outcome, one does not have to stake one's faith in that speculation to justify the research plan. The strategy will be to construct media and devices that offer large surface areas to organisms in soil habitats, but which can be extracted for closer study-- e.g. glass films either bare or with various mineral coatings. These will be manipulated so as to encourage organisms that have a preference for sessile growth on the surface, and then sampled further for organisms that may have a near- or total- dependence on such surfaces. (One may not have to look far; witness algal and cyanophytic films in aquaria; but I do not know of critical comparisons of their growth potential in adherent vs. suspended mode. It is known that the sheathed habit, associated with attachment, of Sphaerotilus natans, is suppressed by high nutrient concentrations -- there may well be many organisms that correspond to the attached phase of Sphaerotilus, being unable to grow except as epibionts.)

The associations of blue-green algae with fissures and voids in the immediate sub-surface of dry Antarctic soils has been pointed out, by E.I. Friedmann, to refute the asserted sterility of this terrestrial habitat, and to point to some caveats in the design of life-detection strategies for planetary exploration. Saprophytic bacteria that would exploit the primary production of the algae would be very difficult to ascertain by conventional methods if they were interface-dependent. This gap in our appreciation of the soil habitat is thus equally important for laying the groundwork for future planetary biological science and engineering. Furthermore, there are many anecdotal observations of the enhanced resistance to thermal sterilization of microbes in soil matrices; but the phenomenon has not been systematically investigated with regard to mechanism. Thus, our projected studies of interface biology bear on problems of survival in hostile environments (terrestrial and extra-terrestrial) as well as upon practical problems of disinfection, e.g. for planetary quarantine.

Cell cultures of mammalian tissues are of course well known to be profoundly affected by their substrates, and by their surface-interactions with one another; but much less has been said about such effects among microbes, notwithstanding the obvious adaptations to surface habitats seen, e.g., among stalked bacteria and in processes like dental decay!

Finally, few students of the origin of life have FAILED to comment on the probable role of clay and other heterogeneous surfaces for the local concentration of chemosynthetic precursors of life. We thus note that, while the central ideas of this research plan are widely entertained, they have only rarely been the subject of concerted investigation; doubtless because of the overwhelming convenience of working with transparent, homogenous media.

Besides the minerals commonly associated with soil, we will also give particular attention to polymeric carbon as an interphase -- the prevalence, and adsorptive and catalytic properties of this material which justify this choice are elaborated in part B. of this proposal.

Bibliography; Part I.

(This is not a systematic reference list, but the following sources will help to illustrate and enlarge upon the ideas just summarized.)

Davies
Ehrlich
Feitelson

Lederberg -- L-forms; Silica Gel preservation
Landmann

Contact inhibition
Alexander
Friedmann
Marshall
Hattori,T, Microbial life in the soil, Dekker, 1973.
SUNDAY, JULY 31, 1977 15:35:00
<<< FILE GROUP:; <SMITH>SRT77,;0

(niho.tmp

A personal note.

I am obliged to record the acute demoralization -- that I know all too well has been experienced by others too -- when the funding for this project was interrupted for six months from fiscal difficulties at the NIAID, and before it was possible for it to be taken up by the NCI, having been impeded by familiar bureacratic obstacles. I had to contemplate whether I should abandon laboratory work in this field; or if not how I would be able to continue financial support for it, for which there are no realistic avenues other than the current application. It is quite possible to get grant/contract funds for alternative lines of work, with purported short-term goals, given but some time: but my own highest priorities continue to be dedicated to the field in which I have worked for the past 30 years, albeit on a steady state scale which is consistent with the time I must also give to other causes. I should note that the Viking mission will have been consummated quite soon, and that it is unlikely there will be any other avenue for

pursuing the study of extra-terrestrial biology-- which is to speak to one source of distraction. Hereinafter too I can detail how it has become possible to integrate my continued interests in computer-science with the present project. And finally, I have to report that while I continue to do some work for W.H.O. and similar agencies, I no longer take the active (and time-consuming role in representing the ideals of science to the public that I did 5 years ago.

J.L.]